Examination of an acid forest soil for ammonia- and nitrite-oxidizing autotrophic bacteria

T. R. HANKINSON AND E. L. SCHMIDT[1]
Department of *Microbiology, University* of Minnesota, Minneapolis, MN, U.S.A. 55455

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An acid forest soil (pH 3.9 – 4.4) from an undisturbed mixed oak stand in southern Indiana was examined for the occurrence of ammonia- and nitrite- oxidizing chemoautotrophs. Populations of both nitrifiers were detected in pH 7 most-probable-number (MPN) autotrophic media, and a Nitrosospira was isolated from highest dilution ammonia oxidizer MPN tubes. Populations of nitrite oxidizers were 10 to 1000 times higher than those of ammonia oxidizers. In pH 4.0 MPN media, ammonia oxidation was slight and unsustainable on 10% transfer to fresh medium, whereas nitrite oxidation was vigorous and sustainable. In pure culture the *Nitrosospira* isolate (Np IO1a) was completely inhibited by nitrapyrin at 5 μg mL^{-1}, tolerant of 1.0 and 10.0 m*M* chlorate, and capable of growth only at pH 6.2 and above. Fluorescent antibodies raised against Np IO1a were used to confirm the predominance of Np IO1a in all MPN series examined. These results suggest that autotrophic ammonia oxidizers may be restricted to circumneutral microsites in this acid soil, whereas autotrophic nitrite oxidizers may not be limited to such sites.

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Les microorganismes de la nitrification autotrophe ont été examinés dans un sol forestier acide (pH 3,9–4,4) sous couvert naturel de chêne, situé dans le sud de l'état d'Indiana. Les populations des bactéries nitrifiantes ont été énumérées par le méthode du nombre le plus probable (MPN) à pH 7 en milieu autotrophe, et une souche Nitrosospira a été isolée à partir des tubes MPN pour les dilutions les plus fortes du ferment nitreux. Les populations des ferments nitriques étaient de 10 à 1000 fois plus nombreuses que celles des ferments nitreux. A pH 4,0, la nitrosation dans les tubes MPN était faible et non soutenue après repiquage à 10% en milieu frais, alors que la nitratation était vigoureuse et pouvait être maintenue. La souche (Nitrosospira Np IO1a) isolée à partir des tubes MPN était totalement inhibée par 5 μg mL^{-1} de nitrapyrine, tolérait le chlorate (1,0 à 10,0 m*M*) et ne pouvait se multiplier qu'à partir d'un pH de 6,2 ou moins acide. Les anticorps fluorescents obtenus à partir de Np IO1a ont confirmé la prédominance de Np IO1a dans tous les tubes MPN. Ces résultats suggèrent que la nitrosation autotrophe aurait lieu autour des microsites neutres de ce sol acide alors que la nitration ne serait pas limitée à ces sites.

Introduction

So far as has been established the chemosynthetic autotrophic nitrifying bacteria are the sole agents of nitrification in natural systems. The nitrification process, however, may proceed at reactions far below the pH limits observed for the nitrifying bacteria in pure culture. Many observations document nitrification at soil pH values around 4.0 (Schmidt 1982), whereas growth of nitrifiers in culture is generally limited to pH 6.5 or higher (Watson 1974). Resolution of this long-standing anomaly has been slow since the microbiology of nitrification in acid environments has received little attention.

Nitrification in highly acid environments must result from the activities of one or more of the following: classical autotrophic nitrifiers active somehow despite their acid sensitivity; acid-tolerant autotrophic nitrifiers; heterotrophic nitrifiers. No acid-tolerant autotrophic nitrifier has been identified, and no heterotrophic nitrifier has been shown to express nitrification in other than pure culture conditions. Strayer et al. (1981) suggested the participation of heterotrophic nitrifiers in artificially acidified forest soil in which they found evidence of nitrification even after the addition of nitrapyrin (2-chloro-6-(trichloromethyl)pyridine), an inhibitor of ammonia oxidation by autotrophic nitrifiers. No microbiological studies were reported.

Chemosynthetic autotrophic nitrifiers, however, have been isolated from certain acid soils capable of nitrification. Bhuiva and Walker (1977) obtained isolates of the ammonia-oxidizing genera *Nitrosospira, Nitrosolobus,* and *Nitrosornonas* from Bangladesh tea soil samples at pH 5.0 – 6.2. Walker and Wickramasinghe (1979) subsequently examined more acidic tea soils (pH 4.0–4.5) from Sri Lanka and Bangladesh. All Bangladesh isolates were species of *Nitrosospira,* while that genus, along with *Nitrosolobus* and one isolate of *Nitrosovibrio,* were isolated from the Sri Lanka soil. In all instances isolations were made at or near pH 7.0 and no data were given on the pH tolerance of the isolates. All isolations were initiated by procedures such that the abundance of the isolate in the natural soil and its possible contribution to soil nitrification could not be assessed. The authors also reported nitrite-oxidizing

[1]Author to whom reprint requests should be addressed.

activity but did not attempt to isolate ***Nitrobacter.*** *Nitrobacter* was isolated from an acid coniferous forest soil (pH 3.5) near Lyon, France by Josserand and Bardin (1981) using the techniques of Walker and collaborators (Bhuiya and Walker 1977; Walker and Wickramasinghe 1979), and pH 7.0 autotrophic media.

The purpose of the present study was to examine the predominant nitrifying population of an acid forest soil for the presence of chemoautotrophic nitrifying bacteria. The soil was obtained from an oak forest site in Indiana. It has been studied extensively as part of an investigation of the effect of forest clear-cutting on nitrogen mineralization and nitrification (Vitousek et al. 1982). This paper emphasizes the characterization of an ammonia-oxidizing isolate. Isolation of predominant nitrite oxidizers is in progress.

Materials and methods

Soil samples

Samples were collected from an undisturbed mixed oak stand in southern Indiana. Nitrification, soil, and vegetation characteristics of the site were described by Matson and Vitousek (1981). Collections were performed with a post-hole digger, and soil samples were transported as intact 12.7 × 16.5 cm columns in 2-lb (1 lb = 0.454 kg) coffee cans. In the laboratory, a portion of the samples was separated by horizon (O, A1, and mineral), and used as inoculum for most-probable-number (MPN) analysis within 1 week of collection. Remaining samples were sealed and stored at room temperature and initial moisture content for subsequent use. Individual soil samples had pH values which fell within the range of 3.8 – 4.4, as measured in a soil – 0.01 M $CaCl_2$ (1:2) suspension.

Most-probable-number estimation of in situ *cell densities*

Enumeration of the ammonia and nitrite oxidizer populations was performed by MPN as described by Schmidt and Belser (1982). Media were adjusted to pH 7 with K_2CO_3 (15%) or to pH 4 with 1 N HCl and were unchanged by autoclaving. Activity in the MPN dilution tubes was monitored using Griess-Ilosvay reagents for NO_2^-, and diphenylamine for NO_3^- (Schmidt and Belser (1982). Media pH did not affect either assay. Since nitrite may decompose in highly acid solutions, we examined this as a potential problem when testing for nitrification in acid media. Nonbiological loss of nitrite was slight to nil at pH 4.5 and above, but increased progressively with decreasing pH below 4.5. Partial decomposition, which slowed after 5 days, was apparent at pH 4.0; nevertheless, uninoculated control and inactive inoculated tubes of nitrite oxidizer medium at pH 4.0 retained readily detectable nitrite throughout incubation periods of up to 4 months. MPN dilution series were monitored for positive end-point dilutions for up to 4 months. During this lengthy incubation period, MPN racks were enclosed in plastic bags to prevent evaporation of the media.

Isolation of an ammonia oxidizer

Attempts were made to isolate from the most abundant nitrifying bacteria in the soil by a nonenrichment approach (Belser and Schmidt 1978*a*). Certain MPN tubes showing activity for ammonia oxidation at the highest dilution were selected as the starting point for isolation. Such tubes were periodically readjusted to pH 7 with sterile 1% K_2CO_3 until roughly 1 m*M* NO_2^- or NO_3^- had been produced. Direct microscopic counts of total bacteria were made using fluorescence microscopy and acridine orange staining of samples on irgalan black treated polycarbonate filters (Schmidt and Paul 1982). Samples were then diluted to 0.5 cell/mL in sterile saline, and 1.0 mL of this cell suspension was inoculated into each of 25 tubes containing 4 mL autotrophic medium. Tubes demonstrating ammonia oxidation following 3 weeks incubation were then screened for purity. Trypticase soy agar (TSA) plates were spread with 0.1 mL from each positive tube, and 1 mL was also transferred to 9 mL sterility medium (Schmidt and Belser 1982). Absence of colonies on TSA and absence of turbidity in the purity test broth after 7 days incubation at 23°C was considered preliminary evidence of a pure culture. Samples from the positive tubes were then examined for purity using phase and ultraviolet (UV) microscopy on acridine orange stained smears. Finally, negatively stained preparations were examined by means of transmission electron microscopy.

Characterization of the *isolate*

The ammonia-oxidizing isolate was characterized for sensitivity to nitrapyrin, chlorate, and acidity. All experiments were carried out in 250-mL flasks containing 100 mL of autotrophic ammonia oxidizer medium. Nitrite production was monitored quantitatively using modified Griess-Ilosvay reagents (Schmidt and Belser 1982). Incubation was at 23°C on a rotary shaker. For the nitrapyrin sensitivity experiment, 2-chloro-6-(trichloromethyl)pyridine (nitrapyrin (N-Serve), lot I5A, Dow Chemical Co., Midland, MI) was prepared as a 20% (w/v) solution in ethanol. Control cultures were amended with an equivalent volume of ethanol minus nitrapyrin. For the chlorate sensitivity experiment, $KClO_3$ was added to individual culture flasks prior to autoclaving. The medium was adjusted to pH 7.0, 6.0, 5.0, and 4.0 with 0.5 or 1.0 N HCl for the initial determination of acid tolerance, and later in the range of 6.0 – 7.2.

Fluorescent antibodies

Production of antiserum and preparation of fluorescent antibody (FA) for the isolate followed the procedures of Belser and Schmidt (1978*b*). Nine other strain-specific FAs, three each for agricultural soil or sewage isolates of Nitrosomonas, Nitrosolobus, and *Nitrosospira*, were selected from previously prepared stocks (Belser and Schmidt 1978*b*), stored at – 40°C as a 1:2 suspension in glycerol. Each FA was tested and titered against its homologous strain, and then combined at appropriate dilution titers to comprise three FA "cocktails," one for each of the three genera. The cocktails were used to screen positive MPN tubes for identifiable serotypes.

Screening of MPN dilution tubes with FA

Unamended samples of Indiana oak soil that had been stored in plastic bags at field moisture capacity and ambient laboratory temperature for about 12 months were used to inoculate an ammonia oxidizer MPN series. The purpose was to confirm the distribution of isolate Np 101a and to screen for other ammonia oxidizers with the FA cocktails. Ammonia oxidation positive

TABLE 1. Most-probable-number analysis of Indiana oak acid forest soil for populations of ammonia and nitrite oxidizers in pH 7.0 media

Sample description	Soil sample	pH	NH_4^+ oxidizers: Cell density (cells/g dry soil)	NH_4^+ oxidizers: C.I. (0.95)* Low	NH_4^+ oxidizers: C.I. (0.95)* High	NO_2^- oxidizers: Cell density (cells/g dry soil)	NO_2^- oxidizers: C.I. (0.95)* Low	NO_2^- oxidizers: C.I. (0.95)* High
0–15 cm depth, composite 1	1a	3.8	3100	939	10230	130000	39400	439000
	1b	3.8	1600	485	5280	70000	21200	231000
0–15 cm depth, composite 2	1c	3.9	1700	515	5610	230000	69700	759000
	1d	3.9	300	91	990	490000	148500	1617000
0–15 cm depth, composite 3	1e	3.8	0	—	—	13000	3900	42900
	1f	3.8	0	—	—	7800	2360	25740
0–15 cm depth, composite 4	2a	4.4	4900	1485	16170	35500	10760	117150
	2b	4.4	5000	1606	17490	12000	3636	39600
Forest floor	3a	4.1	1000	314	3421	43300	13000	142900
Al Horizon	3b	4.4	393	119	1297	10000	3000	33000
Forest floor	3c	3.9	168	51	554	428	130	1400
Al Horizon	3d	4.2	283	86	934	4300	1280	14200
Forest floor	3e	4.3	227	69	749	44000	13400	145000
Al Horizon	3f	4.3	26	8	86	100	30	330

Noie: Samples 1a–1f were collected 12 March 1981 and used as inoculum 19 March 1981; samples 2a–2b were collected 2 September 1981 and used as inuculum 14 September 1981; samples 3a–f were collected 6 July 1982 and used as inoculum 2 June 1983.
*Low and high values, 95% confidence interval (C.I.).

MPN tubes were arnended with an additional 4 mL autotrophic medium and incubated until roughly 500 μM NO_2^- or more cornrnonly NO_2^- had been produced. A 0.5-mL aliquot frorn each tube was applied to each of four irgalan black stained, 0.2 μm pore size, 25 mm diarneter Nuclepore filters (Nuclepore Corp., Pleasanton, CA) (Moawad et al. 1984). Each filter was stained with a single FA preparation; either *Nitrosospira* IOla FA or one of the three NH_4^+ oxidizer FA cocktails. FA staining, washing, and mounting of filter membranes has been described (Schmidt 1974). Filters were examined with a Ziess Standard model 14 microscope, equipped as described by Moawad et al. (1984).

Results

Estimation of chemoautotrophic nitrifiers

The results of the MPN estimation of chemoautotrophic nitrifiers in the lndiana oak forest soil are summarized in Tables 1 and 2. Estimations carried out in autotrophic media at pH 7.0 (Table 1) gave clear evidence for the occurrence of both ammonia and nitrite oxidizers, despite the fact that the ambient pH of the soil was far below that of the MPN media. The enumeration data reflect the high variability that is characteristic of the MPN procedure. Ammonia oxidizer populations were low, ranging to a maximum of only a few thousand per gram of dry soil, but nitrite oxidizer populations were consistently 10 to 1000 times higher. A further indication of the preponderance of nitrite oxidizers was the observation that nearly all tubes positive for ammonia oxidation were detected by the presence of nitrate, rather than nitrite. All of the nitrifier-positive tubes at the highest dilutions (extinction dilutions) were used to inoculate appropriate fresh autotrophic medium, and all maintained nitrifying activity through repeated transfers.

More limited enumerations made in MPN media adjusted to pH 4.0, the approximate pH of the soil, indicated an active population of autotrophic nitrite oxidizers, but no clear indication of autotrophic ammonia oxidizers (Table 2). Nitrite oxidation was rapid and readily maintained through repeated transfer in pH 4 medium. Oxidation of ammonia was observed in scattered tubes at the lowest dilutions, but was very slow, requiring up to 4 months incubation for accumulation of approximately 30 μM nitrite or nitrate. Because activity was not sustained following 10% transfer to fresh medium, it could not be definitely attributed to autotrophic ammonia oxidizers.

Isolation of an ammonia-oxidizing chemoautotrophic nitrifier

One positive ammonia oxidizer tube at the extinction dilution of the pH 7.0 MPN series was selected from each of the subsamples of composite samples 1 and 2 (Table 1) for pure culture isolation. Each of four 25-replicate tube inoculations produced cultures that

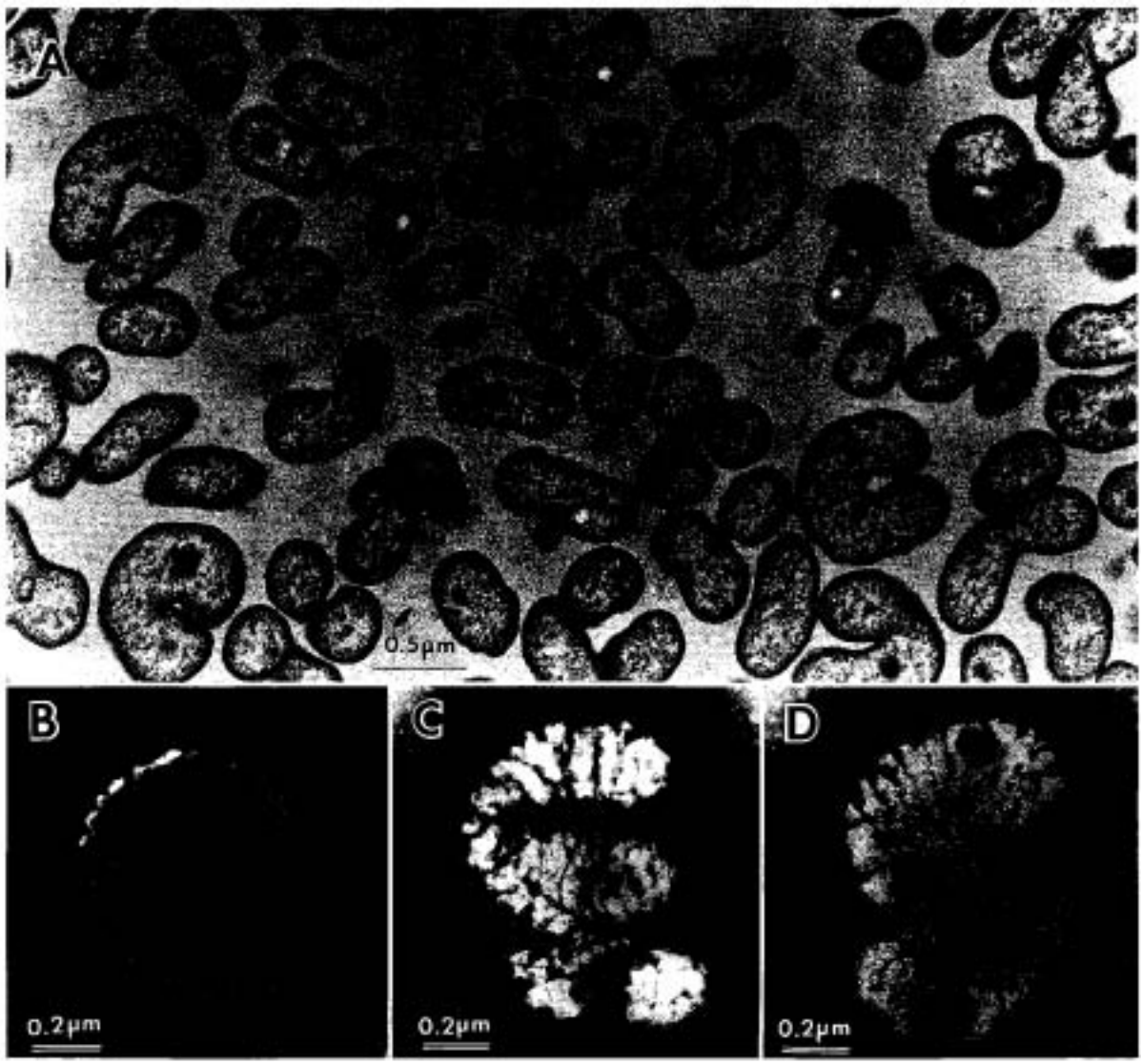

FIG. 1. Electron rnicrographs of *Nitrosospira* IO1a. *(A)* Thin-section preparation. (B–D) Cornrnonly occurring cellular arrangements as seen in negatively stained preparations.

actively oxidized ammonia, ranging from 2 to 11 cultures per 25 inoculated tubes, for a total of 28 active cultures. Two of the 28 cultures, both from MPN series 1a, were judged to be pure and to be identical on the basis of microscopic appearance. The remaining 26 mixed cultures contained cells closely resembling the pure culture in morphology.

The ammonia oxidizer isolate was determined to be a *Nitrosospira* sp. on the basis of light, fluorescence, and electron microscopy and was designated *Nitrosospira* IO1a. The predominant form of the isolate in liquid culture was an individual Gram-negative cell curved in the form of a single 360° spiral. Cell dimensions were approximately 0.3 × 1.3 μm, with the diameter of the spiral about 0.7 μm. Figures 1B – 1D illustrates the cell arrangements observed most frequently in negatively stained electron microscope preparations. Spiral forms longer than the S-shaped spiral (Fig. 1D) were observed occasionally by the light microscopy. *Nitrosospira* IO1a appeared in thin-sectioned preparations (Fig. 1A) commonly as ''U'' and "J" shaped cells due to the plane of sectioning. As seen in Fig. 1A the isolate, in common with other *Nitrosospira* (Watson 1974), lacks the extensive cytomembrane system found in several other genera of autotrophic ammonia oxidizers.

Properties of Nitrosospira *IO1a*

The FA prepared against *Nitrosospira* IO1a gave a

TABLE 2. Most-probable-number analysis of Indiana oak acid forest soil for nitrifying populations in pH 4.0 media

Sample description	Soil sample	pH	NH_4^+ oxidizers: Cell density (cells/g dry soil)	NO_2^- oxidizers: Cell density (cells/g dry soil)	C.I. (0.95) Low	C.I. (0.95) High
0–15 cm depth, composite 4	2*c*	4.4	NR*	351	106	160
	2*d*	4.4	NR	237000	71 850	782500
0–15 cm depth, composite 5	3g	4.1	NR	4000	1 200	13 200
	3*h*	4.1	NR	3000	900	9900
	3*i*	4.1	NR	4000	1 200	13200
	3*j*	4.1	NR	3000	900	9900

NOTE: Samples 2*c*–2*d* were collected 2 Septeniber 1981 and used as inoculum 14 September 1981; samples 3*g*–3*j* were collected 6 July 1981 and used as inoculum 8 March 1983.

*NR, not reliable. Some tubes at lowest (10^{-1}, 10^{-2}) dilutions were positive: however, since activity could not be sustained in passage, data were considered unreliable as a measure of autotrophic nitrifiers.

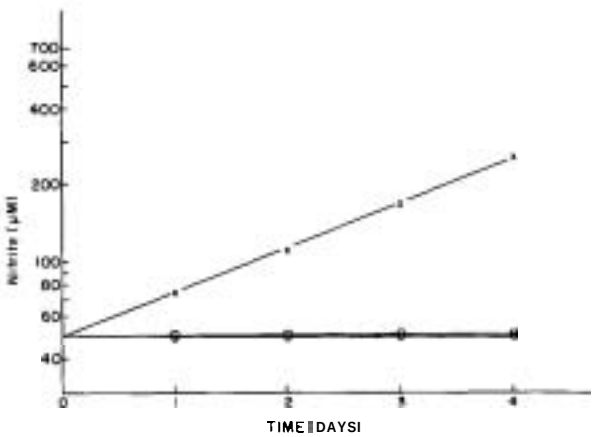

FIG. 2. Effect of nitrapyrin on ammonia oxidation by *Nitrosospira* IO1a. X. No nitrapyrin; □, nitrapyrin (5 μg mL^{-1}); O. nitrapyrin (10 μg mL^{-1}).

3+ (Belser and Schmidt 1978*b*) homologous reaction when diluted 1:4 in saline and a 2+ cross-reaction with 4 of 17 *Nitrosospira* strains from the laboratory culture collection. Serological relatedness among the five cross-reactive strains has not been studied as yet. Strains represented in the FA cocktails (see Materials and methods) did not cross-react with the Np IO1a FA.

The sensitivity of the isolate to nitrapyrin, an inhibitor of autotrophic ammonia-oxidizing genera, was normal (Belser and Schmidt 1981). An exponentially growing culture was completely inhibited by 5 μg nitrapyrin/mL culture (Fig. 2). Response of the isolate to chlorate, a chemical used to inhibit nitrite oxidation in the short-term nitrification assay (Belser and Mays 1980), was also typical of ammonia oxidizers. The isolate was tolerant of 1.0 and 10.0 m*M* chlorate; in fact, these concentrations appeared to stimulate activity slightly (Fig. 3). A concentration of 100 mM chlorate decreased the rate of oxidation by 93%.

The acid tolerance of *Nitrosospira* IO1a was of particular interest since it was isolated (at pH 7.0) from a pH 3.8 soil. In a preliminary growth experiment to assess acid tolerance, the isolate grew at pH 7.0, but not at pH 6.0 or lower. A subsequent experiment at various pH levels between pH 6.0 and 7.2 is summarized in Fig. 4. The lower limit of acid tolerance in pure culture was about pH 6.2, and a preference for increasingly alkaline growth circumstances was clear.

FA examination of MPN tubes for ammonia oxidizers

A total of 28 MPN tubes, including the highest ammonia oxidation positive dilutions of Indiana oak soil samples 3*a*–3*f* (Table 1), was screened for the presence of ammonia oxidizers reactive to *Nitrosospira* IO1a-FA or to any of nine other FAs representative of serotypes of the genera *Nitrosomonas, Nitrosolobus,* and *Nitroso spira.* Data presented in Table 3 show that *Nitrosospira* IO1a was detected in 20 of the 23 dilution tubes in which ammonia oxidation had occurred. These included some of the lower, as well as the highest, positive dilutions. Two nitrification-positive tubes also contained cells which were reactive against the *Nitrosomonas* FA mixture (IO3b, 10^{-1}; IO3d, 10^{-2}). Further examination of these two tubes with individual FAs revealed that the cross-reactivity was to antiserum raised against two strains of *Nitrosomonas* isolated from sewage.

Discussion

Enumeration of nitrifier populations by the MPN rnethod is an indirect, statistical approach which is time-

TABLE 3. Nurnber of actively nitrifying MPN dilution tubes or inactive (control) tubes containing FA-reactive bacteria per total number of MPN tubes examined

MPN series*	MPN dilution	Np lO1a	Ns cocktail?	NI cocktail	Np cocktail
lO3a	10^{-2}	2/2	0/2	0/2	0/2
	10^{-3}	2/2	0/2	0/2	0/2
103b	10^{-2}	2/2	0/2	0/2	0/2
	10^{-3}	1/1	1/1	0/1	0/1
	10^{-4}	1/1	0/1	0/1	0/1
lO3c	10^{-2}	4/4	0/4	0/4	0/4
lO3d	10^{-2}	4/4	1/4	0/4	0/4
	10^{-3}	2/2	0/2	0/2	0/2
103e	10^{-2}	1/3	0/3	0/3	0/3
	10^{-3}	0/1	0/1	0/1	0/1
103f	10^{-2}	1/1	0/1	0/1	0/1
lO3e control‡	10^{-2}	0/1	0/1	0/1	0/1
	10^{-4}	0/1	0/1	0/1	0/1
103f control	10^{-3}	0/1	0/1	0/1	0/1
	10^{-3}	0/1	0/1	0/1	0/1
	10^{-4}	0/1	0/1	0/1	0/1

*See Table 1 for description of samples used for MPN series inoculations.
†Each cocktail is a mixture of three FAs. Ns = *Nitrosomonas* strains ATCC. SE. and Sewage: NI = *Nitrosolobus* strains *AV*, Fargo. and Bearden: Np = *Nitrosospira* strains *AV*, Spitz-30. and Spitzbergen. See Belser and Schmidt (1978*b*) for description of strains.
‡Control tubes were inoculated but gave no evidence of ammonia oxidation.

consuming, inherently lacking in precision (Cochran 1950; Taylor 1962), and is selective as a function of medium composition (Belser and Schmidt 19786). Data provided by MPN analyses, however, allow for comparisons among samples and rough approximations of populations and were particularly useful in the context of this study as a means of distinguishing a numerically dominant segment of nitrifiers for the purposes of examination and pure culture isolation. It is clear from the results of MPN enumeration that both ammonia- and nitrite-oxidizing autotrophs occupied the Indiana acid forest soil. Identification of Nitrosospira lO1a in highest-dilution MPN tubes by means of its FA suggests it was the most abundant autotrophic ammonia oxidizer occupying this soil. The occurrence of some cells reactive to two Nitrosornonas FAs, however, and some nitrification-positive tubes without FA-reactive cells indicate that Np lO1a was not the only ammonia oxidizer.

Identification of the isolate as a member of the Nitrosospira genus lends further association of this genus with nitrification in terrestrial systems. Belser and Schmidt (1978*a*) found Nitrosospira to be the predominant genus of ammonia oxidizers in a field soil, and the isolates most commonly obtained from acid

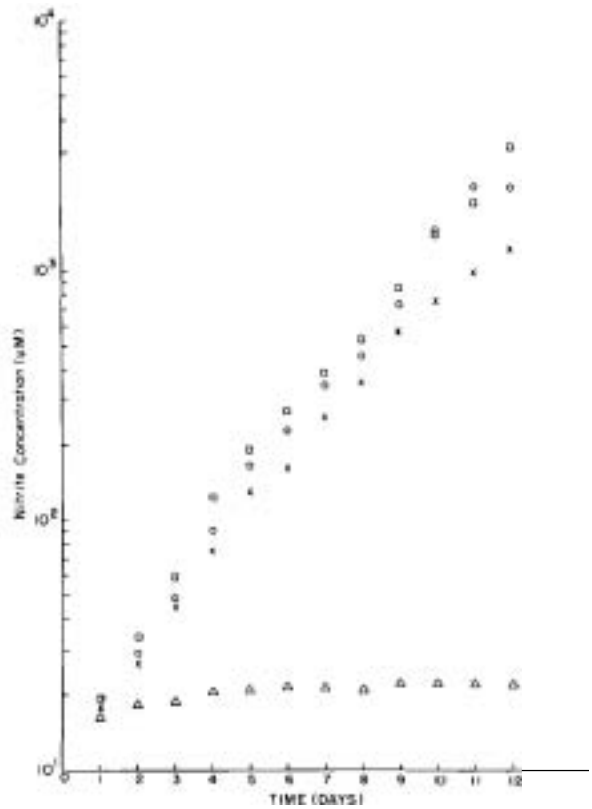

FIG. 3. Effect of chlorate on the arnmonia-oxidizing activity of isolate *Nitrosospira* lO1a. X, No chlorate; ○, 1.0 m*M* $KClO_3$; □, 10.0 m*M* $KClO_3$; A, 100 m*M* $KClO_3$.

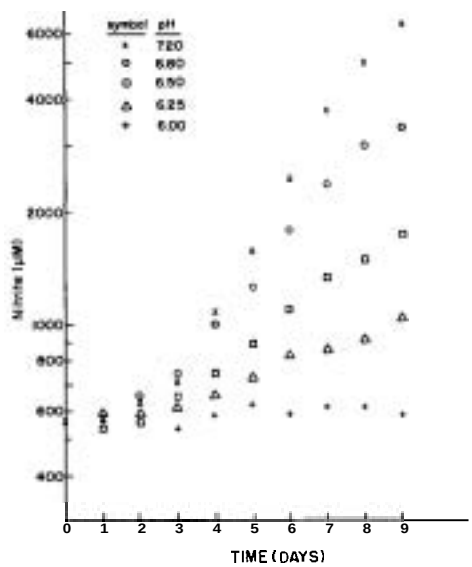

FIG. 4. Influence of pH on the growth of isolate *Nitrosospira* lO1a. The pH was monitored daily; initial pH values remained unchanged.

soils by Bhuiya and Walker (1977) and Walker and Wickramasinghe (1979) were also described as *Nitrosospira*. *Nitrosospira* IOla isolated from a highly acid forest soil in the course of this study closely resembled the description and photomicrographs of the *Nitrosospira* isolated by Walker and Wickramasinghe (1979) from a Bangladesh acid tea soil. The predominant form of *Nitrosospira* IOla, also like that of the Bangladesh isolate, was a single spiral. These apparently differ from *Nitrosospira briensis* which occurred primarily in a multispiral arrangement (Watson 1971). Watson (1971) reported the occurrence of small forms of *Nitrosospira briensis* but as a small fraction of the population.

Just how the observed acid sensitivity of *Nitrosospira* IOla can be reconciled with its occurrence as a predominant autotrophic nitrifier in a highly acid soil raises a question of broad significance to microbial ecology, i.e., the importance of the microsite to microbial activity in the natural environment. If the isolate contributes significantly to nitrification in the acid forest soil as is apparently the case, it must occur in fairly abundant acid-protected microsites in the soil. It has long been recognized that the pH values obtained by the usual techniques to measure soil acidity represent averages based on deliberate homogenization of the sample, whereas pH values within an undisturbed sample vary substantially from point to point (Kubiena 1938). Further investigations based on the "niche" hypothesis, particularly as it relates to nitrifying populations, are obviously needed.

The highest population densities of nitrite oxidizers relative to ammonia oxidizers in samples of the acid forest soil is unusual, although not unprecedented (Belser 1977), in natural environments. These data suggest that organisms other than chemosynthetic autotrophic ammonia oxidizers may be responsible for some fraction of the net nitrite produced in this soil. Alternatively, some nitrite may be derived from nitrate reductions (Belser 1977). A third possibility is that some nitrite oxidizers as facultative autotrophs may grow mixotrophically with reduced dependence on nitrite. In any event, nitrite oxidation activity was significant and sustainable in MPN medium at pH 4.0, as well as pH 7.0, indicating that the nitrite-oxidizing population, in contrast to the population of ammonia oxidizers, may not be restricted to acid-protected microsites. Attempts to isolate and characterize components of the nitrite-oxidizing populations of this highly acidic soil are in progress.

Acknowledgements

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